

**APPENDICES
FOR
UNITED STATES LETTERS PATENT**

**TITLE: OVERCOMING DAPA AMINOTRANSFERASE BOTTLENECKS
 IN BIOTIN VITAMERS BIOSYNTHESIS**

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2 PAGES OF APPENDICES

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Appendix I. Medium composition for biotin and vitamers production in bench scale fermentors.

Medium Component	Batch	Concentration	Feed
Glucose		15.0 g/liter	750 g/liter
Veal Infusion Broth ¹		25.0 g/liter	---
Yeast Extract ¹		5.0 g/liter	---
Sodium Glutamate		5.0 g/liter	---
KH ₂ PO ₄		7.5 g/liter	13.7 g/liter
MgCl ₂ ·6H ₂ O		1.0 g/liter	1.5 g/liter
(NH ₄) ₂ SO ₄		2.0 g/liter	---
MAZU DF-37C		2.5 g/liter	---
CaCl ₂ ·2H ₂ O		1.0 g/liter	4.0 mg/liter
CuSO ₄ ·5H ₂ O		0.4 mg/liter	5.0 mg/liter
ZnSO ₄ ·7H ₂ O		0.5 mg/liter	35.0 mg/liter
MnSO ₄ ·H ₂ O		25.0 mg/liter	10.0 mg/liter
FeSO ₄ ·7H ₂ O		1.0 mg/liter	2.0 mg/liter
Sodium Molybdate-2H ₂ O		0.2 mg/liter	100.0 mg/liter
Sodium Citrate-2H ₂ O		50.0 mg/liter	100.0 mg/liter

¹ In Amberex Medium the Veal Infusion Broth and Yeast Extract are replaced with 10 g/l Amberex 695.

Appendix II. Protocol of avidin-HABA [2-(4-hydroxyphenylazo) benzoic acid] displacement assay for biotin and dethiobiotin.

Reagents and Solutions:

Buffer: 0.1 M NaPO₄, pH 7.0.
Avidin: From Sigma (Cat # A-9275). Dissolved at 5 mg/ml in Buffer.
HABA: From Aldrich (Cat # 14,803-2). Dissolved at 0.375 M in water +
1 eq. NaOH.

Prepare Mix:

	20 samples	50 samples
Avidin	1 ml	2.5 ml
HABA	0.08 ml	0.2 ml
Buffer	38.9 ml	97.3 ml

Assay:

Zero spectrophotometer;

Add 2 ml of Buffer to disposable 5 ml cuvette; record OD₅₀₀.

To read sample:

Place disposable 5 ml cuvette in spectrophotometer.

Add 2 ml of Mix; stir; record OD₅₀₀.

Add sample in 0.1 ml volume; stir; record OD₅₀₀.

Standards:

Use 0.1 ml DTB at 2 mg/ml to 14 mg/ml as samples.

Use 0.1 ml Buffer as "zero" point.

Calculations:

Calculate ΔOD₅₀₀ minus ΔOD₅₀₀.

Plot standards and use curve to determine HABA vitamers from samples.

- Notes: 1. Useful range is 2 to 14 mg/l of biotin + dethiobiotin.
2. Add mix to cuvette, read OD₅₀₀, and then add sample and mix without removing cuvette from the spectrophotometer.
3. Best results are obtained when a constant volume is used with a set of samples and standards. Use Buffer to bring all samples to the same volume.